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14. ABSTRACT

Osteoblastic bone metastasis is a common complication of advanced prostate cancer, resulting in pain and pathologic fracture. Dickkopf homolog 1 (DKK1) is a secreted inhibitor of osteoblast Wnt signaling pathway and hypothesized to be a central regulator of prostate cancer osteoblastic bone metastasis. The purpose of this proposal is to examine mechanisms of DKK1 regulation by prostate cancer cells and determine whether DKK1 overexpression in bone blocks the formation of osteoblastic bone lesions in animal models of bone metastasis. We have now shown that human prostate cancer cell lines that produce osteolytic, but not osteoblastic, bone lesions in animal models of bone metastasis express significant amounts of DKK1 and this expression is correlated with the absence of DNA methylation at the DKK1 promoter CpG island. Our preliminary data points to a central role of DKK1 in prostate cancer bone metastasis and expect this work to translate into the development of novel therapeutic targets to treat this malignancy complication.

15. SUBJECT TERMS
Prostate cancer, Bone metastasis, Dickkopf homolog 1, Wnt signaling

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INTRODUCTION

Osteoblastic bone metastasis is a common complication of advanced prostate cancer, resulting in pain and pathologic fracture (*1*). In mouse models and human clinical studies of prostate cancer, tumor-produced endothelin-1 (ET-1) activates the osteoblast endothelin A receptor and increases new bone formation (*2*). In previously published work from our group, we demonstrated that dickkopf homolog 1 (DKK1), a negative canonical Wnt signaling regulator, is reduced by ET-1 resulting in enhanced canonical Wnt signaling activity and new bone formation (*3*). Others have shown that DKK1 secretion from prostate cancer cells themselves also contribute to bone microenvironment DKK1 (*4*). We hypothesized that DKK1 is a central regulator of prostate cancer bone metastasis. The purpose of this proposal is to examine mechanisms of DKK1 regulation by prostate cancer cells and determine whether DKK1 overexpression in bone blocks the formation of osteoblastic bone lesions in animal models of bone metastasis. Understanding the role of DKK1 in bone metastasis will facilitate the development of modulators of this factor and other Wnt signaling members. The development of such novel and targeted therapies to bone would represent a significant advancement in the treatment of prostate cancer metastasis to bone.

BODY

The PI moved from the University of Virginia to the University of Alabama at Birmingham on November 6, 2009. Because of expected delays in research, a no-cost extension was granted for a new end date of 2/28/2012. The last report submitted July 1, 2010 was a partial annual report. The current report represents data generated since the last report (10 month period).

Task 1: Determine if the osteoblastic response to ET-1 is blocked by *Dkk1* transgenic overexpression targeted to bone in mouse models of prostate cancer bone metastasis

In Task 1, *Dkk1*^{Ob} mice in the C57BL/6 genetic background were to be bred to C57BL/6 nude animals. However, it remained unclear whether human cancer cells commonly used in mouse metastasis models (BALB/c nude) form bone lesions similarly in the C57BL/6 background. In addition since mice carrying the nude mutation are poor breeders, the SCID mutation (*Prkdc*^{scid}) was a preferred immunodeficient model. A first pilot experiment was performed as stated in last year's report which showed the tumor take of the LuCaP23.1 xenograft was 9/10 for BALB/c nude mice and 1/10 for C57BL/6 nude mice (Fisher's exact test; p=0.0011). Because of these results, the cells for the osteoblastic bone metastasis model were changed to ARCaPm prostate cancer cells. This recently available prostate cancer cell line has the advantage over LuCaP23.1 xenograft cells in that they grow in culture, form osteoblastic bone lesions in both athymic nude and SCID mice, and form lesions more rapidly within 8 weeks after intratibial injection.

The viability of ARCaPm cells in mice carrying the SCID mutation in the C57BL/6 genetic background was recently tested. Tumor take was 6/6 for BALB/c nude vs. 1/6 for C57BL/6 SCID. Based on the low tumor appearance in the C57Bl/6 backgrounds, mice in the BALB/c background will be used. A modified breeding strategy has been implemented using the same number of mice indicated in the original protocol.

Round 1: *Dkk1*$^{Ob+/-}$ (C57BL/6) X WT (BALB/c)
F1 ½ ***Dkk1*$^{Ob+/-}$ (mixed)**
½ *Dkk1*$^{Ob-/-}$ (mixed)

Round 2: *Dkk1*$^{Ob+/-}$ (mixed) X *Prkdc*$^{scid/scid}$ (BALB/c)

F2 ½ ***Prkdc*$^{scid/WT}$*/Dkk1*$^{Ob+/-}$**
½ *Prkdc*$^{scid/WT}$*/Dkk1*$^{Ob-/-}$

Round 3: *Prkdc*$^{scid/WT}$*/Dkk1*$^{Ob+/-}$ X *Prkdc*$^{scid/scid}$ (BALB/c)
F3 ¼ ***Prkdc*$^{scid/scid}$*/Dkk1*$^{Ob+/-}$**
¼ ***Prkdc*$^{scid/scid}$*/Dkk1*$^{Ob-/-}$**
¼ *Prkdc*$^{scid/WT}$*/Dkk1*$^{Ob+/-}$
¼ *Prkdc*$^{scid/WT}$*/Dkk1*$^{Ob-/-}$

This modified breeding protocol will ensure that the study mice will possess at least 87.5% of the BALB/c background to maximize the number of mice that accept tumor. Round 1 is currently in progress and is expected that this Task will be complete by the 2/28/2012 end date.

Task 2: Determine how DKK1 production from bone cells and tumor is regulated *in vivo* in osteoblastic bone metastasis

The work proposed in this task is dependent on Task 1 and will be performed concurrently.

Task 3: Determine if *Dkk1* is inactivated by promoter CpG island hypermethylation in prostate cancer

Work continues on this Task focused on 1) correlating DKK1 expression of prostate cancer cells with behavior in bone (osteoblastic vs. osteolytic) and 2) uncovering mechanisms of DKK1 expression by promoter methylation. The following data detailed in the last report is summarized as follows:

1. Prostate cancer cell lines that produce osteoblastic/mixed lesions in animal models of bone metastasis secrete more DKK1 than the PC3 osteolytic cancer cell line.

2. DKK1 promoter methylation correlates with DKK1 expression.

3. Demethylation restores DKK1 expression osteoblastic/mixed prostate cancer cell lines.

4. In the PC3 prostate cancer cell line with large DKK1 expression, Wnt signaling is still active.

5. Kremen, the DKK1 receptor, is downregulated in PC3 cells suggesting a mechanism for DKK1 resistance.

We recently examined whether Kremen overexpression in PC3 cells restores DKK1 sensitivity and Wnt responsiveness. PC3 cells were transfected with a Kremen overexpressing vector along with canonical Wnt signaling reporter vectors. With Kremen overexpression, Wnt signaling significantly decreased (Figure 1).

Future studies include examining whether Kremen overexpression in PC3 cells changes *in vitro* behavior (proliferation or apoptosis) or the bone phenotype in an animal model of bone metastasis.

KEY RESEARCH ACCOMPLISHMENTS

- Osteolytic, but not osteoblastic, prostate cancer cells express DKK1

- Osteolytic, but not osteoblastic, prostate cancer cells have unmethylated DKK1 promoter
- Demethylation corrects DKK1 suppression
- Low Kremen (DKK1 receptor) blocks Wnt suppression from high DKK1 in PC3 prostate cancer cells
- Kremen overexpression in PC3 prostate cancer cells partially restores DKK1 inhibition of canonical Wnt signaling.

REPORTABLE OUTCOMES

Invited Oral Presentation, DoD IMPaCT 2011 Meeting, Orlando
"Regulation of prostate cancer bone metastasis by the Wnt signaling inhibitor dickkopf homolog 1"

Abstract:
Background and Objectives: Normal bone homeostasis and remodeling are disrupted with the arrival of metastatic prostate cancer cells to bone. Through cooperative and cross-talking signals that foster the formation of bone metastasis, bone provides a hospitable microenvironment for the invading cancer cells. In turn, prostate cancer cells manipulate existing pathways that stimulate the osteoblast to form pathologic bone. One factor secreted by prostate cancer cells is endothelin-1 (ET-1). ET-1 binds to the osteoblast endothelin A receptor and activates pathologic bone formation. This occurs through downregulation of the secreted Wnt signaling inhibitor dickkopf homolog 1 (DKK1). DKK1 is also a product of prostate cancer cells. We hypothesized that bone microenvironment DKK1, a product of both osteoblasts and prostate cancer cells, is a central regulator of the osteoblastic response to metastatic prostate cancer.
Brief Description of Methodologies: DKK1 expression analysis was performed using promoter and 3' UTR reporter vectors and ELISA. DNA methylation of the DKK1 promoter was determined by methylation-specific sequencing.
Results to Date: Mechanisms of DKK1 regulation by osteoblasts and prostate cancer cells were examined. ET-1 rapidly reduced osteoblast DKK1 message and protein secretion, but did not alter *Dkk1* promoter activity in murine primary osteoblasts or RNA PolII promoter occupancy. However, ET-1 destabilized *Dkk1* mRNA through a mechanism dependent on the *Dkk1* 3' UTR. Putative RNA binding elements and species conserved miRNA consensus sequences within this long 3' UTR regulated by ET-1 are being examined.

DKK1 regulation in prostate cancer cells was also investigated. A CpG island is positioned within the promoter and first exon of *DKK1*. Methylation of CpG islands is often dysregulated in cancer and contributes to misexpression of genes and to tumorigenesis. Progressive *DKK1* promoter hypermethylation and transcriptional downregulation may in fact occur during prostate cancer bone metastasis. *DKK1* expression and CpG island methylation was examined in prostate cancer cell lines. C4-2B, C4-2 and LnCaP prostate cancer lines exhibited low DKK1 expression while PC3 and DU145 prostate lines had significant *DKK1* expression. Methylation-specific sequencing of the *DKK1* CpG island was performed on the human cancer cells. Hypermethylation at the *DKK1* promoter correlated with the lowest DKK1 expression in cancer cell lines. *DKK1* expression was restored with the DNA demethylating agent 5-aza-2'-deoxycytidine, confirming that epigenetic mechanisms regulate its expression.

Conclusions: Osteoblast activity and pathologic bone formation are regulated by DKK1, a product of both prostate cancer and osteoblasts. The mechanisms involve both static epigenetic promoter silencing and dynamic control of *DKK1* mRNA stability via its 3' UTR.
Impact statement: The majority of cancer therapies are aimed at systemic eradication of cancer cells but tailored therapies focusing on the treatment of specific cancers that metastasize to particular organs and tissues are the future of cancer therapy. DKK1 represents a promising novel therapeutic target for prostate cancer bone metastasis. Delayed pathologic bone formation may in fact allow increased penetration of conventional cytotoxic agents so the that in the future prostate cancer bone metastasis may be cured with combination therapies.

CONCLUSION

Bone metastasis is a significant complication of advanced prostate cancer that causes pain and pathologic fracture. This work is aimed at uncovering the role of DKK1 in prostate cancer bone metastasis. We have discovered a correlation between behavior of prostate cancer in bone, DKK1 expression and DNA methylation of the DKK1 promoter. We will extend this work and examine DKK1 promoter methylation patterns in human prostate cancer bone metastasis and whether this pattern correlates with risk for and progression of bone metastasis. Studies that are in progress will examine whether overexpression of DKK1 in the bone microenvironment blocks bone metastasis in an animal model. Medical and social costs of bone metastasis are high. This work is expected to translate into improved treatments for prostate cancer bone metastasis and facilitate the development of therapeutic targets to DKK1.

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APPENDICES

None

SUPPORTING DATA

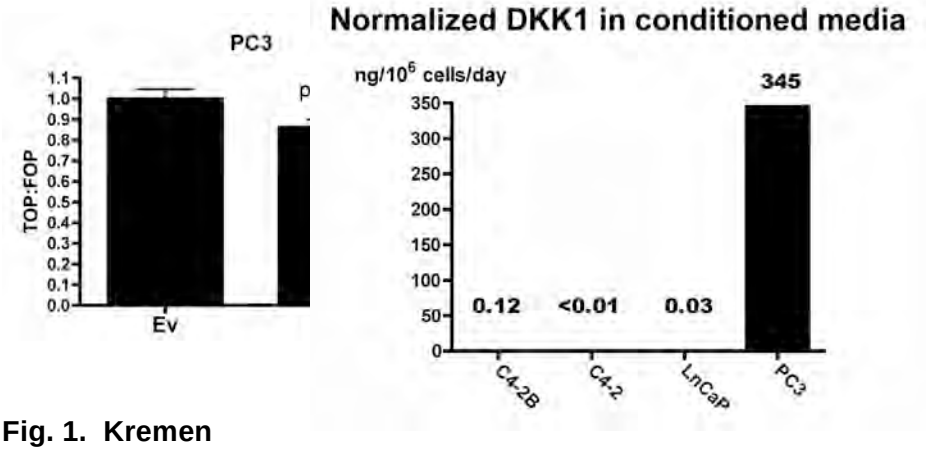

Fig. 1. Kremen overexpression in PC3 cells reduces canonical Wnt signaling.